

Near- and Mid-Infrared Spectroscopy

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Comparison of Near- and Mid-Infrared Spectroscopy for Herb and Spice Authenticity Analysis

Introduction

Food authenticity and adulteration testing has taken on a new level of importance with consumers and producers, after recent food adulteration scandals. Food fraud negatively impacts consumer confidence

and, more importantly, some adulteration can be harmful to health. Herbs and spices are one of the most commonly adulterated commodity products, with a prime example being oregano adulterated with olive and myrtle leaves¹.

Infrared (IR) spectroscopy is well recognised for the characterisation of a wide range of materials and has advanced significantly over the last few decades². This fast and cost-effective technique, when combined with a spectral library, can provide conclusive information about samples, and is ideally suited to both qualitative analysis of materials and quantification of components. Historically, IR instruments were generally quite large, required clean environments, and highly trained operators for interpretation of results. Advancements in sampling techniques, data analysis tools, ruggedness, and instrument portability have allowed easy analysis of a broad range of samples within a range of environmental settings³.

IR spectroscopy combined with chemometric techniques such as Principal Components Analysis (PCA) is an extremely useful technique for food analysis. This application note compares the use of Mid- and Near-IR spectroscopy for authenticity analysis and adulteration detection in herbs and spices.

Infrared Spectroscopy

The IR region of the spectrum is generally split into three different sub regions:

- Far-IR: 400 – 30 cm^{-1}
- Mid-IR: 4000 – 400 cm^{-1}
- Near-IR: 14000 – 4000 cm^{-1}

The Far-IR region is primarily used for measuring inorganic molecules, thus not relevant for herb and spice analysis⁴. The Mid-IR region consists of fundamental absorption bands and provides a molecular fingerprint. This is especially beneficial for simple structural investigation and raw material ingredient or additive identification by library comparison. Absorption bands in the Near-IR region arise from overtones and combination bands of the fundamental bands in the Mid-IR region. The Near-IR region is particularly useful for food applications, including moisture, fat and protein content determination.

Comparing Mid- and Near-IR Spectroscopy for Herb and Spice Analysis

Foods are typically mixtures and encompass numerous sources of natural variation. In many cases in particular herbs and spices, IR spectroscopy combined with chemometric techniques such as PCA, which accommodate variation, can be used for sample classification.

The most common sampling techniques for food samples are Attenuated Total Reflectance (ATR) for Mid-IR and diffuse reflectance for Near-IR. Diffuse reflectance may be used with Mid-IR but absorptions would be too strong without sample


dilution. Table 1 compares Near- and Mid- IR methods for herb and spice analysis.

As a surface technique, ATR is highly suited to liquids and bulk homogeneous material measurement, but is less satisfactory for trace analysis, such as adulterant detection in herbs and spices. In diffuse reflectance mode, Near-IR radiation can penetrate much further into the sample, generating a long effective pathlength, yielding stronger spectra, thus making it more effective for adulterant detection analysis.

Mid-IR with ATR uses a small sample area and the resulting reproducibility of sampling is inconsistent due to pressure differences and different sample contact. Sample homogeneity of herbs and spices is typically poor, resulting in variations in spectra from different portions of the same sample. For leaf based materials, ATR generally analyzes a portion of a single leaf, making it unlikely to detect adulteration in the samples unless they are homogenised. Although Near-IR with diffuse reflectance requires much larger sample volumes, the large sampling area and ability to spin the sample during a scan results in better spatial averaging and more reproducible and representative sampling.

Figure 1 shows the Mid- and Near-IR spectra of ten different portions of a sage sample. Figure 2a-2c shows the standard deviation spectra for sampling different portions of the sample in un-powdered and powdered form. Given that a flat baseline in the standard deviation spectrum would represent no variance, large variance was observed between sampling in the Mid-IR spectra of the un-powdered form. Although less variance was observed when sampling powdered sage using Mid-IR (Figure 2b), it was still greater in comparison with the Near-IR standard deviation spectrum (Figure 2c). This demonstrates that homogenisation is a required sample preparation step for measuring herbs and spices by ATR in Mid-IR spectroscopy. Comparing Figures 2a and 2c (the Mid- and Near-IR standard deviation spectra of the un-powdered sage), it is evident that there is significantly lower variance using Near-IR sampling.

Table 1. Near- and Mid-IR comparison for herb and spice analysis.

	Near-IR	Mid-IR
Sampling Type	Diffuse reflectance (non-destructive)	ATR (non-destructive)
Sampling Size	 <p>Large (60-100 mm across)</p>	 <p>Small (2 mm across)</p>
Sample Quantity	Several grams (generally not an issue as plenty of sample is usually available)	Homogeneous liquids and powders/solids
Sample Requirements	High	Poor for inhomogeneous materials but high for liquids
Sampling Consumables	Glass petri dishes – transmit Near-IR radiation, are relatively low-cost, and no accessory cleaning is required	Cleaning with solvent and tissue
Sample Pathlength	~100 μm to several millimetres	A few microns (μm)

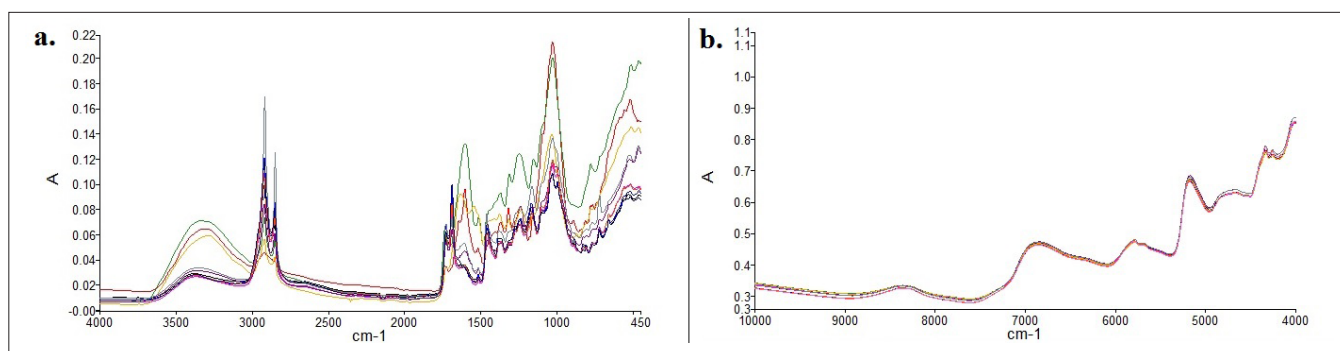


Figure 1. Ten different portions of the same sage sample: (a) Mid-IR spectra (b) Near-IR spectra.

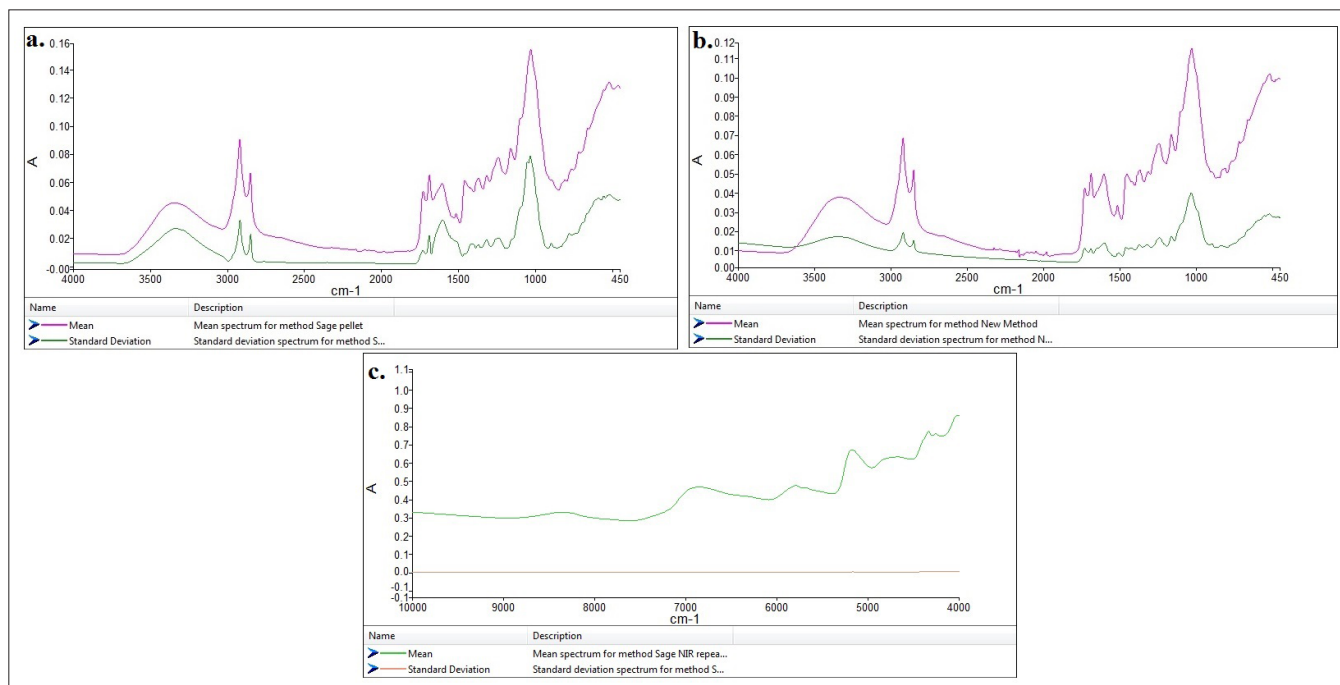


Figure 2. Mean and Standard Deviation of (a) Mid-IR spectra of unhomogenized sage; (b) Mid-IR spectra of powdered sage; (c) Near-IR spectra of unhomogenized sage.

Adulterant Screen Methods for Near-IR and Mid-IR

PerkinElmer's Adulterant Screen™ is an algorithm, using PCA, specifically designed for screening raw materials and products to determine authenticity, identify adulteration and estimate its levels⁵. It involves creating a spectral library of unadulterated samples for any given material. This library should include most of the possible sources of natural variation which arise from different suppliers, batches, sample pre-treatments and geographical origins. A library of pure adulterants is also required, but with just one spectrum for each adulterant. PerkinElmer Spectrum® Touch methods can readily be created for the Adulterant Screen methodology which encompasses a simple user interface; thus enabling routine operators to achieve rapid results for authenticity testing.

Twenty samples of sage and four samples of other materials (thyme, oregano, basil, and chives) were obtained from The Bart Ingredients Company (Bristol, UK). A spiked sample of 10% (w/w) thyme in sage was prepared to demonstrate the method. All samples were run on a PerkinElmer Frontier™ FT-IR instrument in both Near- and Mid-IR. Wavenumber ranges for Near- and Mid-IR spectra were 10,000 - 4000 cm⁻¹ and 4000 - 450 cm⁻¹ respectively. Scanning parameters were set to 8 cm⁻¹ resolution

and a one minute scan time. Separate Adulterant Screen Touch methods were created for each wavenumber range, using pure sage as the material spectra, and the other materials as adulterant spectra. After creating the Adulterant Screen methods, the 10% thyme in sage spiked sample was run four times, in Near- and Mid-IR, and the average thyme (%) levels estimated.

Adulterant Screen results for the 10% thyme in sage spiked sample are shown in Figure 3 for the Near-IR method. All samples failed Adulterant Screen, indicating adulteration. The average thyme level (%) was calculated to be 12.00%, illustrating reasonable levels of accuracy for this complex natural product, and the estimated detection limit was determined to be 10.29%. These detection limit orders of magnitude are expected with plant based materials, which are spectrally quite similar due to their cellulose content. However, adulteration with dissimilar materials would result in detection at lower concentrations. The 10% thyme in sage was not detected by the Mid-IR adulterant screen method. A potential explanation for this is the small ATR sampling area results in analyzing only a few flakes of the sample, making it problematic for inhomogeneous materials, and illustrating the difficulties in adulterant detection using Mid-IR spectroscopy.

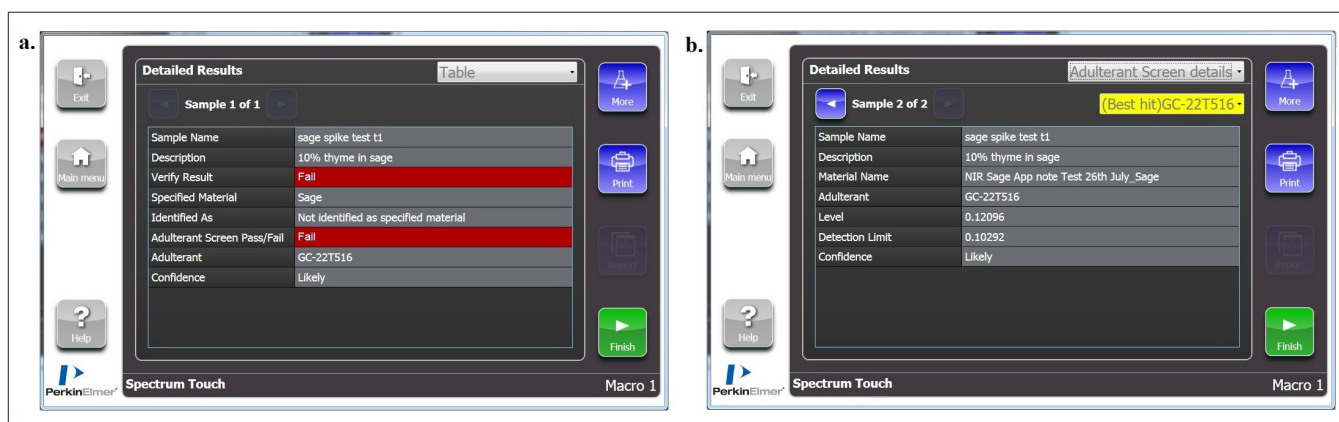


Figure 3. (a) Touch method results for 10 % thyme in sage sample for Near-IR (b) Detailed Adulterant Screen Touch results where GC-22T516 is thyme.

Conclusion

There is considerable scope for rapid on-site analysis of foods by using the combination of IR spectrometers with PCA to classify complex materials. This application note has shown that Near-IR spectroscopy is more suitable than Mid-IR for the analysis of herbs and spices and could also be applied to other inhomogeneous foods.

Near- and Mid-IR are both non-destructive techniques requiring no sample preparation. However, the longer effective pathlength and better spatial averaging available with Near-IR diffuse reflectance is beneficial for adulterant detection, and using glass sample containers allows for rapid analysis with no accessory cleaning required. Larger sampling areas and the ability to spin the material during Near-IR analysis results in more reproducible and representative sampling of food products with an inhomogeneous nature. Thus Near-IR spectroscopy is the ideal solution for herb and spice authenticity and adulteration detection analysis.

References

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